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TOPICAL REVIEW

Genes and elite athletes: a roadmap for future research

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Abstract There is compelling evidence that genetic factors influence several phenotype traits related to physical performance and training response as well as to elite athletic status. Previous case-control studies showed that ∼20 genetic variants seem to be associated with elite endurance athletic status. The present review aims to introduce novel methodological approaches in the field of sports genetics research, which can be applied in the near future to analyse the genotype profile associated with elite athletic status. These include genotype–phenotype association studies using gene expression analysis, analysis of post-transcriptional factors, particularly micro-RNAs, genome-wide scan linkage or genome-wide association studies, and novel algorithm approaches, such as 'genotype scores'. Several gaps in the current body of knowledge have been indentified including, among others: small sample size of most athletic cohorts, lack of corroboration with replication cohorts of different ethnic backgrounds (particularly, made up of non-Caucasian athletes), the need of research accounting for the potential role of epigenetics in elite athletic performance, and also the need for future models that take into account the association between athletic status and complex gene–gene and gene–environment interactions. Some recommendations are provided to minimize research limitations in the field of sport genetics.

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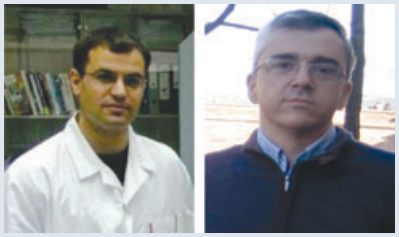
Introduction

Genetic factors influence several phenotype traits related to sports performance and elite athletic status ('elite athlete refers to one who has competed at a national or international level in a given sport'; Macarthur & North, 2005). The main question is no longer whether there is a genetic component associated with elite athletic status and endurance/power trainability, but rather, which genetic profiles contribute to elite performance. For instance,



Nir Eynon (left) earned his Masters degree from Zinman College, Wingate, Israel and his PhD degree with distinction from Porto University, Portugal. He is currently a postdoctoral Fellow in the Department of Nutritional sciences, Ariel University center, Israel, under the supervision of Dr Ruth Birk. His main areas of research at the moment are (i) genetics of exercise with emphasis on elite athletic performance and (ii) nutriogenetics, the effect of enviromental factors on the genome. **Alejandro Lucia** (right) is an MD and PhD, and a professor of Exercise Physiology in the *Universidad Europea de Madrid*, Spain. His current main areas of research are (i) genetics of exercise and (ii) benefits (and involved mechanisms) of exercise prescription for diseased and special populations (children and adults with cancer, children with cystic fibrosis, adolescents with anorexia nervosa, pregnant women and elderly). He also works extensively with McArdle (glycogenosis V) patients.

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~66% of the variance in athlete status is explained by additive genetic factors, with the remaining variance being attributable to non-shared environmental factors (De Moor *et al.* 2007). Current and future research will elucidate the elite athletic-related genetic profile and the mechanisms and signal pathways involved.

More than a decade ago, Montgomery and colleagues identified for the first time a positive association between a genetic variation, the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene (*ACE*), and endurance exercise performance (Montgomery *et al.* 1998). They found that the I allele and the II genotype were overrepresented in experienced British high-altitude mountaineers (with a history of ascending beyond 7000 meters without using supplementary oxygen) compared to their healthy, non-athletic referents. This pioneer research highlighted the need for a clear phenotype definition. Follow-up studies, such as the GENEATHLETE project carried out by Prof. Bouchard's group managed to provide a robust phenotype for elite athletic status by using a genuine world-class group of Caucasian athletes with a maximum oxygen uptake ($\dot{V}_{O_2max}$) of at least 75 ml kg^{-1} min^{-1} (Doring *et al.* 2010*a*,*b*,*c*). The latest 'human gene map for performance and health-related fitness phenotypes' identified more than 200 genetic variations potentially associated with physical performance phenotypes or training responsiveness, yet only about 20 polymorphisms were found to be specifically associated with elite athletic status (Bray *et al.* 2009). By definition elite athletic phenotype is a complex one; thus, it is likely that the effect of a single gene variant is small. As a consequence, there is a need to use more comprehensive approaches to identify the 'optimum' genotype profile for endurance and power-oriented phenotypes and trainability (Bouchard *et al.* 2010; Rankinen *et al.* 2010). This review presents several novel methodological approaches in the field of sports genetics research, which can be applied in the near future to analyse the genotype profile associated with elite athletic status.

Genotype–phenotype association studies using gene expression analysis

Phenotype linked to gene expression analysis strategy uses a 'reverse' approach to DNA variation. In this method, athletic performance heterogeneity is identified by RNA expression studies, leading to validation and identification of the physiologically relevant DNA variation and theoretically to increased statistical power. The HERITAGE family study group applied this innovative approach to identify a molecular classifier that predicts the training responsiveness of $\dot{V}_{O_2max}$ in non-athletes (Timmons *et al.* 2010). In this study, two independent pre-intervention RNA expression data sets were generated from 41 subjects who underwent supervised endurance training. Twenty-nine RNA signatures were found to predict $\dot{V}_{O_2max}$ training response out of which six new single nucleotide polymorphisms (SNPs) were found to be positively associated with gains in $\dot{V}_{O_2max}$. Generating the RNA predictor also led this group to discover almost 50% of the estimated genetic variance for the $\dot{V}_{O_2max}$ gains following aerobic training in humans.

If applied to elite athletes, the aforementioned methodological approach could accelerate the discovery of genetic biomarkers associated with elite athletic performance. A disadvantage of analysing gene expression at the skeletal muscle level is that this method can only identify the athletic performance heterogenetic profile related to genes expressed in human skeletal muscle fibres; this approach is nevertheless of interest due to the fact that nearly 50% of the variance of slow-twitch (type I) muscle fibre proportion seems to be determined by genetic factors (Simoneau & Bouchard, 1995). The peroxisome proliferator-activated receptor α gene (*PPARA*) intron 7 G/C polymorphism (rs4253778) is associated with muscle fibre type composition in young men, i.e. GG homozygotes had significantly higher percentages of type I fibres (55.5 ± 2.0 *vs.* $38.5 \pm 2.3\%$, $P = 0.003$) than CC homozygotes (Ahmetov *et al.* 2006). Ahmetov *et al.* (2009) screened 10 common metabolic gene polymorphisms including the *PPARA* 7G/C variation, associated with elite endurance athlete status, and concluded that the number of endurance-related alleles was associated with the proportion of fatigue-resistant, type I fibres, as well as with endurance performance, and with $\dot{V}_{O_2max}$. Taken together, these findings suggest that the potential influence of the *PPARA* 7G/C polymorphism on endurance performance might be partially explained by the association between *PPARA* genotypes and muscle fibre type composition.

An extensively studied gene polymorphism in athletes is the α-actinin-3 (*ACTN3*) Arg(R)577Ter(X) polymorphism (rs1815739). The expression of the skeletal muscle protein α-actinin-3 is almost exclusively restricted to fast twitch (type II) muscle fibres (Mills *et al.* 2001), where it constitutes one of the major components of the Z-disc. α-Actinin-3 stabilises the muscle contractile apparatus, which may confer a higher capacity for force absorption/transmission compared to type I fibres (Mills *et al.* 2001). This protein might also promote the formation of type II fibres. Indeed, sarcomeric α-actinins bind the calsarcins (Frey *et al.* 2000; Frey & Olson, 2002), which interact with calcineurin, a signalling factor that plays a role in the specification of muscle fibre type (Serrano *et al.* 2001). More than a billion people worldwide cannot express α-actinin-3 in their skeletal muscle fibres (i.e. they are homozygous for the R577X null-allele) (MacArthur & North, 2004). It has been demonstrated that the *ACTN3*

knockout mouse (α-actinin-3 deficient) has a decreased activity in the anaerobic glycolytic pathway and increased activity in the aerobic oxidative pathway (MacArthur & North, 2007). Furthermore, *ACTN3* knockout mice also exhibit higher fatigue resistance, decreased muscle mass and fibre diameter of fast (IIB) twitch muscle fibres, and reduced muscular strength compared to wild-type mice (MacArthur *et al.* 2007, 2008).

In humans, Yang *et al.* (2003) showed that Olympic women finalists in 'power' or 'sprint' events (jumping, throwing, 100-metre running) rarely exhibit the 'null' XX genotype of the *ACTN3* R577X polymorphism (Yang *et al.* 2003). With some exceptions (Lucia *et al.* 2007; Druzhevskaya *et al.* 2008), these findings were replicated in a number of studies with elite sprint/power athletes (Niemi & Majamaa, 2005; Papadimitriou *et al.* 2008; Roth *et al.* 2008; Santiago *et al.* 2008; Eynon *et al.* 2009*a*; Massidda *et al.* 2009).

Post-transcriptional factors: role of microRNAs (miRNAs)

microRNAs (miRNAs) are non-coding RNA molecules of an average length of 22 nucleotides that act mainly as post-transcriptional regulators of protein synthesis. miRNAs play a role in the regulation of metabolism (Esau *et al.* 2004; Esau *et al.* 2006), and they are associated with disease phenotypes, such as insulin resistance in type II diabetes (Gallagher *et al.* 2010). miRNAs might also regulate skeletal muscle post-transcriptional gene expression, and thus modulate important aspects of muscle function, including its contractility (van Rooij *et al.* 2008). The potential role of miRNAs in the response to both endurance and strength training was recently reported (Gallagher *et al.* 2010; Timmons *et al.* 2010; Davidsen *et al.* 2011; Keller *et al.* 2011). Keller *et al.* (2011) conducted a miRNA profiling of sedentary subjects and showed that several miRNAs targeting RUNX1, SOX9 and PAX3 were down-regulated by endurance training. Davidsen *et al.* (2011) showed that individual variations in resistance training-induced gains in skeletal muscle mass were associated with selected changes in miRNA abundance: miR-378, miR-29a and miR-26a were down-regulated in low responders and unchanged in high responders, whereas miR-451 was up-regulated only in low responders (Davidsen *et al.* 2011).

Elucidating the role of miRNAs in the phenotypic change and individual variability in response to exercise training represents a promising area for further investigation in the field of genetics and athletic performance.

Genome-wide scan linkage or genome-wide association studies

Genome-wide scan linkage or genome-wide association studies allow the analysis of polymorphic markers of the whole genome combined with informatics and robust statistics to link genetic markers to physiological phenotypes. De Moor and colleagues (2007) were the pioneers in genome-wide scan linkage studies related to athletic status. They studied 4488 British adult monozygotic and dizygotic female twins and estimated that the heritability of athletic status was 66% (De Moor *et al.* 2007). In a more recent genome-wide association study, the same group reported that the 3′-phosphoadenosine-5′-phosphosulfate synthase 2 gene (*PAPSS2*) was associated with exercise participation (pooled P values $<1.0 \times 10^{-5}$) in a cohort of 1644 unrelated Dutch and 978 unrelated American adults (De Moor *et al.* 2009). A genome-wide association study recently conducted by Bouchard *et al.* (2010) identified a set of 21 SNPs accounting for 49% of the variance in $\dot{V}_{O_2max}$ trainability (Bouchard *et al.* 2010). The strongest association with the training response of $\dot{V}_{O_2max}$ was found to be an acyl coenzyme A synthetase long-chain 1 gene (*ACSL1*) polymorphism (rs6552828), which accounted for 6% of the training response of $\dot{V}_{O_2max}$. However, it is noteworthy that their main findings were different from those identified in the same population using another method to predict the genetic component of $\dot{V}_{O_2max}$ trainability, i.e. analysis of RNA expression 'signatures' at the muscle level (Timmons *et al.* 2010). Thus, there is an obvious need for replication studies using different approaches, populations and exercise training regimens.

Predictive algorithms ('genotype scores')

Using bioinformatical approaches for identifying the individual and combined contribution of a group of genetic variants to elite athletic status, Williams & Folland (2008) determined the probability for the existence of humans with a theoretically 'optimal' polygenic profile for endurance sports (Williams & Folland, 2008). They quantified such 'optimal' profiles using a 'genotype score' (ranging from 0 to 100, with '0' and '100' being the worst and best genotype combinations, respectively), a simple algorithm resulting from the best accumulation combination of 23 candidate polymorphisms explaining individual variations in endurance performance. The polygenic profile was computed assuming (i) an additive effect, and (ii) the fact that all gene variants were given the same weight in the total score. However, whether the selected genes and the gene variants explain the same proportion of the variance in any complex trait is not known. They predicted that the probability of a Caucasian individual existing in the

planet with a 'perfect' genotype score is extremely low (0.0005%), which indicates that there would be approximately three such individuals in the United Kingdom (population ∼60 million). Using a similar model, yet limited to seven well studied polymorphisms associated with endurance capacity in Caucasians (*ACE* I/D (rs1799752); *ACTN3* R577X (rs1815739); adenosine monophosphate deaminase 1 (isoform M) gene (*AMPD1*), Gln(Q)12Ter(X) (rs17602729); creatine kinase, muscle gene (*CKMM*) *Nco*I RFLP 1170bp/985 + 185bp; hereditary haemochromatosis gene (*HFE*) His(H)63Asp(D) (rs1799945); myostatin (growth and differentiation factor) gene (*GDF-8*) Lys(K)153Arg(R) (rs1805086); and peroxisome proliferator-activated receptor-γ, coactivator 1, α gene (*PPARGC1A*) Gly(G)482Ser(S) (rs8192678)), we determined the genotype score of 46 Spanish male world-class elite athletes who were either Olympic finalists (5000 m to marathon) or Tour de France finishers (Ruiz *et al.* 2009) (Fig. 1). We observed that the genotype score of the endurance elite athletes group was significantly higher than for the Spanish population (70.2 ± 15.6 *vs.* 60.8 ± 12.1, respectively), suggesting an overall more 'favourable' polygenic profile in the former. It should be noted that none of the study elite world-class athletes had the optimal genotype score (i.e. 100), and only three of the best Spanish endurance athletes (who were also amongst the best in the world) had the best possible score for up to six polymorphisms (genotype score of ∼93). More interestingly, a top-three finisher in the Tour de France had only three endurance-related genotypes, with a genotype score of ∼57. This theoretical model was applied in Israeli national and international level athletes using six candidate polymorphisms related to mitochondrial biogenesis, that is polymorphisms in the *PPARGC1A*–nuclear respiratory factor (*NRF*)–mitochondrial transcription factor A (*TFAM*) pathway (Eynon *et al.* 2011*a*). In addition, we recently computed a genotype score (*ACE* I/D; *ACTN3* R577X; angiotensinogen gene (*AGT*) Met235Thr (rs699); *GDF*-8 K153R; interleukin-6 gene (*IL6*) 174 G/C (rs1800795); and nitric oxide (NO) synthase gene (*NOS3*) −786T>C (rs2070744)) for power elite athletic status (Fig. 2; Ruiz *et al.* 2010). The general conclusion of the aforementioned studies was that, despite the possibility of a given individual possessing a theoretically 'optimal' athletic polygenic profile being very small, overall the genotype score approach might help in distinguishing elite endurance athletes both from the general population

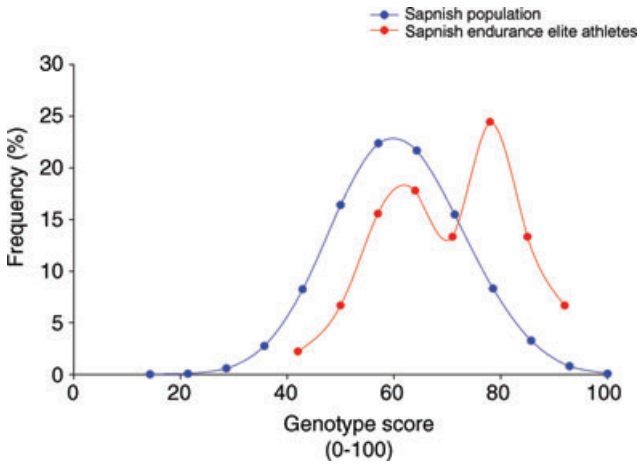

Figure 1. Frequency distribution of genotype scores (0–100) obtained from a theoretical model sample of 50,000 randomly selected Spanish individuals, and from actual data of 46 world-class Spanish endurance athletes
The genotype score was computed with seven polymorphisms (assuming an additive effect) associated with endurance capacity in Caucasians: angiotensin converting enzyme gene (*ACE*) insertion(I)/deletion(D) (rs1799752); α-actinin-3 gene (*ACTN3*) Arg(R)577Ter(X) (rs1815739); adenosine monophosphate deaminase 1 (isoform M) gene (*AMPD1*) Gln(Q)12Ter(X) (rs17602729); creatine kinase, muscle gene (*CKMM*) *Nco*I RFLP 1170bp/985 + 185bp; hereditary haemochromatosis gene (*HFE*) His(H)63Asp(D) (rs1799945), myostatin (growth and differentiation factor) gene (*GDF-8*) Lys(K)153Arg(R) (rs1805086); and peroxisome proliferator-activated receptor-γ, coactivator 1, α gene (*PPARGC1A*) Gly(G)482Ser(S) (rs8192678). Adapted from Ruiz *et al.* (2009).

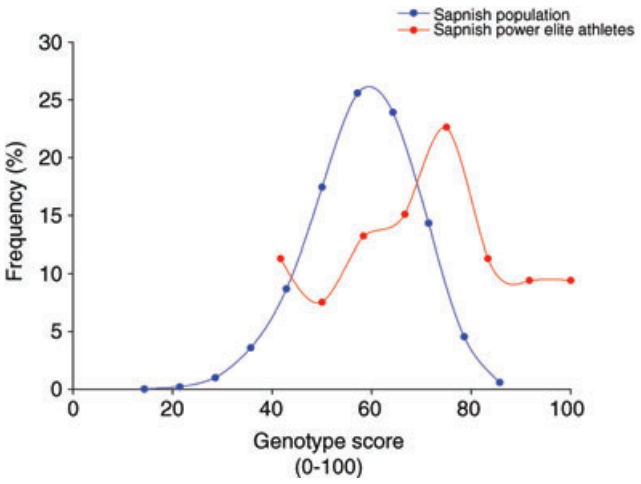

Figure 2. Frequency distribution of total genotype scores derived from a model sample of 50,000 randomly selected Spanish individuals, and from actual data of 53 elite Spanish power athletes
The genotype score was computed with six polymorphisms (assuming an additive effect) associated with power capacity in Caucasians: angiotensin converting enzyme gene (*ACE*) insertion(I)/deletion(D) (rs1799752); α-actinin-3 gene (*ACTN3*) Arg(R)577Ter(X) (rs1815739); angiotensinogen gene (*AGT*) Met235Thr (rs699); myostatin (growth and differentiation factor) gene (*GDF-8*) Lys(K)153Arg(R) (rs1805086); interleukin-6 gene (*IL6*) 174 G/C (rs1800795); and nitric oxide (NO) synthase gene (*NOS3*) –786T>C(rs2070744). Adapted from Ruiz *et al.* (2010). Adapted from Ruiz *et al.* 2010 (*J Appl Physiol*, American Physiological Society, used with permission)

(Ruiz *et al.* 2009; Eynon *et al.* 2011*a*) and from elite power athletes (Ruiz *et al.* 2010). These findings are further confirmed by a recent study conducted in 1423 Russian athletes, which showed that the proportion of subjects with a high number ($\geq$9) of endurance-related alleles was significantly higher in the best endurance athletes compared with controls (85.7 *vs.* 37.8%) (Ahmetov *et al.* 2009).

It is noteworthy that the power of the genotype score approach is entirely dependent on the following factors: (i) analysis and polymorphisms included in the score, (ii) weightings given to each genotype, (iii) likelihood of having the best score being allele dose dependent, (iv) study population, and (v) outcome variable. Future algorithms should consider the inclusion of potential candidate polymorphisms that considered individually may not be associated to elite status, but in interaction with other polymorphisms could have a main effect on the outcome.

Research gaps in the field and recommendations

The first methodological limitation in the field lies on the fact that genotype:phenotype studies need very large population samples (i.e. hundreds or thousands) to reach sufficient statistical power to allow making solid conclusions. It is difficult to reconcile this premise with the scarce number of athletic champions worldwide for a given ethnicity and sport event. Between-study differences in the competition level of athletes, sex and ethnic group further complicate this issue. With regards to the latter issue, there is a clear need to replicate association results between genetic polymorphisms and athletic status in populations of different ethnic backgrounds. For instance, the association that our group found between elite power athletic status and the *IL6* −174 G/C polymorphism in a Caucasian (Spanish) cohort was not corroborated in Israeli Caucasians (Eynon *et al.* 2011*b*). Conversely, the association that we previously reported between the functional C825T polymorphism (rs5443) in the human guanine nucleotide binding protein β protein polypeptide 3 (*GNB3*) gene in Israeli elite athletes (Eynon *et al.* 2009*b*) was not corroborated in Spaniards (Ruiz *et al.* 2011). Also, the current knowledge on genetic factors associated with human exercise phenotypes comes mainly from Caucasian populations; as such, more research is needed with other ethnic groups. An additional potential gap to be kept in mind when interpreting the literature in the field is that studies reporting no genetic association with athletic status (i.e. 'negative results') are less 'attractive' and thus less likely to be published, than others showing 'statistical significance' ('positive results').

As for population selection, some studies report data on athletes of mixed sport disciplines, i.e. not clearly 'endurance' or 'power' oriented, thereby involving phenotypic heterogeneity. Ideally, studies in the field should provide data from athletes who are at the two end-points of the human sports performance continuum, i.e. power events (e.g. sprinting, jumping, throwing, weightlifting) *vs.* endurance events (e.g. marathon running, road cycling or long distance triathlons), or alternatively, clearly describe the phenotype of the specific sport. This would provide a better picture of the alleles associated with either power or endurance elite athletic status. Indeed, the phenotype traits that determine performance in both types of events are likely to be different, and so should be the polygenic profiles. Garland *et al.* (1990) suggested that individuals are inherently predisposed toward sprint or endurance performance.

Studies investigating gene expression analysis are strongly recommended to better understand the molecular mechanisms underlying the association between a given polymorphism and exercise performance phenotypes. Nonetheless, ideally this would require collecting samples from different tissues (e.g. skeletal muscles, myocardium), which might not be feasible in many human subjects, not to mention elite athletes. Thus, much of what we already know on sports genetics and will learn in the future has to be inferred from studies in non-athletic populations, such as the participants from the HERITAGE family study (Timmons *et al.* 2010).

As for predictive algorithms such as the genotype score, several new candidate polymorphisms will likely appear in the foreseeable future, allowing for more accurate predictions. It is nonetheless difficult to account for all the candidate genetic polymorphisms that are potentially associated with elite athletic status. There are indeed numerous other contributors to the 'complex trait' of being an athletic champion that are not likely to be reducible to defined genetic polymorphisms, e.g. technique, kinematics, motivation, pain tolerance. Athletic success is also influenced by 'external' factors that are totally independent from genetic endowment (e.g. social support, or economic possibility).

Finally, future research might determine to what extent the changes that environmental factors can induce in gene expression during critical periods of prenatal and postnatal development (i.e. through epigenetic mechanisms; Waterland & Michels, 2007) explain why some individuals reach the elite athletic status. For instance, elite Kenyan runners, who have dominated most distance running events in the last two decades, undergo stringent training regimens since childhood (running $\sim$20 km day^{-1}) at high altitude ($\sim$2000 m) (Saltin *et al.* 1995), which might lead to unique environment–gene interactions. Future research should also consider models that take into account potential complex interactions between genetic variants, that is gene–gene interactions, including those interactions between genetic variants that might not influence endurance performance

individually. Furthermore, multiple rare alleles may also be able to explain the likelihood of being an elite athlete. More studies are also needed investigating the functional significance of new candidate polymorphisms. For instance, a recent study performed in Han Chinese elite runners reported that two intronic polymorphisms of the calcineurin genes were associated with elite athletic status; this study also provided some evidence for a functional significance of these polymorphisms using a luciferase reporter construct (He *et al.* 2011).

Finally, studies in the field of sport genetics will gain credibility in the overall area of genetics if they adhere to the recommendations for human genotype–phenotype association studies recently published by the NCI-NHGRI Working Group on Replication in Association Studies (Chanock *et al.* 2007). These recommendations include, among others, that 'a subset of notable polymorphisms should be evaluated with a second technology that verifies the same result with excellent concordance'.

In summary, up-to-date research data have indicated that genetic endowment has a significant influence on sports performance and on the potential to become an athletic champion; several new methodological approaches have recently been applied, and should be used in the near future to identify the genetic profile that enables attainment of elite athletic status. The main limiting factor for future studies in the field is to recruit large enough samples of elite athletes (i.e. World or Olympic class) of different ethnic backgrounds. Thus, large collaborations and data sharing between research centres worldwide are strongly recommended.

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